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ISOLATED METAPHASE CHROMOSOMES STABILIZED BY DNA-INTERCALATION OR POLYAMINE ADDITON: A COMPARISON

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Interest in the isolation of metaphase chomosomes has increased by the recent application of flow cytometry to chromosome suspensions to detect aberrations or to sort specific chromosomes. A most critical aspect of chromosome isolation procedures is the stabilization of chromosomes upon release from the cells. A recent procedure includes addition of polyamines to the surrounding medium for stabilization (1,2). In a very simple procedure we use DNA-intercalators for the same purpose (3-5). Here, we compare the morphological and biochemical characteristics of chromosomes stabilized by these methods, as well as their suitability to flow karyotyping.

The intercalated chromosomes were much longer than those stabilized by polyamines. Using a Chinese hamster cell line, DON Wg3-h, which contains one easily recognizable subtelocentric chomosome we measured the length of this chomosome after isolation, fixation on a slide, and air-drying. Dependent on the intercalator, this resulted in a 26% to 76% difference between intercalated chromosomes and those isolated with polyamines (Fig. 1).

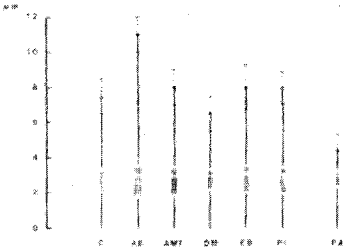

Fig. 1. Length measurements (mean ±S.D., n=20) of a specific chromosome isolated in the presence of the intercalators adriablastin (AB), a psoralen derivative (AMT), daunomycin (DM), ethidium bromide (EB), and propidium iodide (PI), and of the same chromosome isolated in the presence of polyamines. After isolation the chromosomes were fixed on slides. As a control (C) the same chromosome fixed within cells was measured.

The less condensed appearance of the intercalated chromosomes did certainly not detract from their suitability for flow karyotyping. Irrespective of the isolation procedure, a very good resolution of peaks in the flow histogram was obtained (Fig. 2).

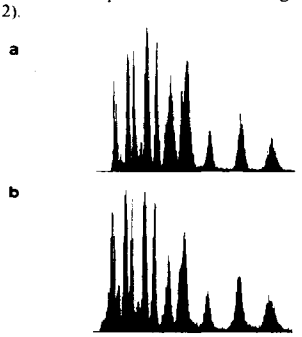

Fig. 2. Flow karyograms of chromosomes isolated in the presence of the intercalator propidium iodide (a) or in the presence of polyamines (b) from V79 Chinese hamster cells.

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DNA extracted from samples of chromosomes isolated by each of both methods and subsequently electrophoresed in agarose appeared to be about 100 kb long, (Fig. 3). Patterns of chromosomal proteins resulting after SDS-polyacrylamide electrophoresis, fixation, and silver staining were very similar with the exception of a few specific bands. The propidium iodide-chromosomes gave an extra band at about MW 27,000 which was absent in the pattern from the polyamine-chromosomes, whereas the latter gave extra bands at MWs 33,000 and 52,000 (Fig. 4).

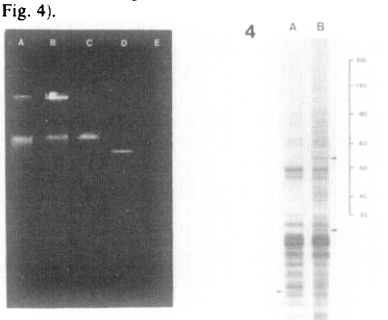

Fig. 3. Size determination of DNA extracted from chromosomes isolated in the presence of the intercalator propidium iodide (A) or in the presence of polyamines (B) in 0.25% agarose. For comparison DNA from bacteriophages H1 (120 kb) and lambda (49 kb), and an Eco R1 digest from the latter DNA (largest fragment 23 kb) were coelectrophoresed (C, D, and E, respectively).

Fig. 4. Silver-stained pattern of chromosomal proteins extracted from chromosomes isolated in the presence of propidium iodide (A) or polyamines (B) after electrophoresis in a 10% SDS-polyacrylamide gel. Molecular weights ×10⁻³.

In conclusion, both methods are equally suitable for the isolation of metaphase chromosomes to be used for biochemical studies. Although they can also both be applied very successfully for flow karyotyping, the intercalation method should be preferred because - in contrast to the polyamine method - it has been shown to be compatible with banding of the chromosomes allowing the direct identification of the vast majority of them (4,5).

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